Circadian Variation in Plasma 17-Hydroxyprogesterone in Patients with Congenital Adrenal Hyperplasia

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Atherden, S. M., Barnes, N. D., and Grant, D. B. (1972). Archives of Disease in Childhood, 47, 602. Circadian variation in plasma 17-hydroxyprogesterone in patients with congenital adrenal hyperplasia. Plasma 17-hydroxyprogesterone (17-OHP) levels in 4 patients with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency were greatly raised and showed a marked circadian variation, with high morning levels and much lower values during the late evening. This finding indicates that patients with CAH may require relatively little adrenal suppression during the late evening and early hours of sleep, and suggests that the main suppressive dose of steroid should be reserved for the period between 3.00 a.m. and 3.00 p.m.

In the majority of patients with congenital adrenal hyperplasia (CAH), the defect in steroid biosynthesis appears to be due to deficiency of 21-hydroxylase, an enzyme necessary for conversion of 17-hydroxyprogesterone (17-OHP) to 11-desoxy-cortisol. As a result of this deficiency, excessive amounts of 17-OHP are secreted by the adrenal glands and the plasma levels of 17-OHP in children with CAH may be 50–200 times higher than the values found in normal men (Strott, Yoshimi, and Lipsett, 1969). This paper describes results obtained in 4 patients with CAH which indicate that there is marked circadian variation in plasma 17-OHP in this condition.

Patients and Methods

Patients. Four patients with CAH due to 21-hydroxylase deficiency were studied. The first (Case 1), a boy aged 4 years with the simple virilizing form of the disorder, was investigated before any treatment was begun. Two other patients who were not salt-losers were studied 3 days after treatment had been temporarily discontinued. One was a 14-year-old boy (Case 2) who had been treated with steroids since the age of 6. He had received 5 mg prednisone twice daily for several months before the period of investigation and his urinary 17-oxosteroid excretion had ranged from 7·7 mg/24 hr to 9·3 mg/24 hr on this treatment. The other was a 16-year-old girl (Case 3) who had been treated since the age of 1 year. Before investigation she had received 7·5 mg cortisol acetate three times a day and the levels of her urinary 17-oxosteroids lay between 6·5 mg/24 hr and 7·6 mg/24 hr. The fourth patient (Case 4), an 18-year-old girl with the salt-losing form of the disorder, was investigated while receiving 25 mg cortisone acetate every 6 hours. Before the period of investigation she had been treated with 50 mg cortisone acetate twice daily and though she showed no gross evidence of virilization, her persistent amenorrhoea suggested that her adrenal hyperplasia was poorly controlled. Her urinary 17-oxosteroid excretion was 26·4 mg/24 hr.

Methods. Venous blood samples were obtained at 11.00 p.m. and 9.30 a.m. in Case 1. Serial blood samples were obtained at roughly 2-hour intervals for 24 hours in the other 3 patients, using an indwelling intravenous catheter.

Plasma 17-OHP was estimated by a simple protein-binding technique (Barnes and Atherden, 1972) based on the method of Murphy (1967). Plasma samples were extracted with 2% ethanol in petroleum ether. Tritated cortisol and dilute human plasma (CBG) were used to estimate the 17-OHP extracted. Using 0·05 ml volumes of plasma, the lower limit of sensitivity of the assay is 0·5 µg/100 ml plasma and the assay is insufficiently sensitive to detect the low levels of plasma 17-OHP found in normal subjects (Strott et al., 1969; Abraham et al., 1971). Details of the precision of the assay have been given elsewhere (Barnes and Atherden, 1972).

The method is not specific for 17-OHP as progesterone is also extracted from plasma. However, as progesterone shows only a 40% competition for CBG when compared with 17-OHP, and as the plasma progesterone

Received 3 January 1972.
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Results

The results obtained in Case 1 before treatment was started are given in the Table. The plasma 17-OHP level at 11.00 p.m. was 0.7 µg/100 ml, but a second specimen taken at 9.30 a.m. the following morning gave a value of 13.8 µg/100 ml.

**TABLE**

<table>
<thead>
<tr>
<th>Plasma 17-OHP Progesterone (µg/100 ml)</th>
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<td>2300 hours</td>
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<td>0930 hours</td>
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The results of serial determination of plasma 17-OHP on the remaining 3 patients are shown in the Fig. The two patients who were not receiving treatment showed a fourteenfold to eighteenfold variation in plasma 17-OHP over a 24-hour period. Both patients had relatively low values between 10.00 p.m. and midnight, but by 3.00 a.m. the levels had risen considerably. In Case 2 a peak level of 23 µg/100 ml was obtained at 9.00 a.m., but in Case 3 fairly consistent values around 9 µg/100 ml were found between 5.00 a.m. and 11.00 a.m. In both subjects the plasma 17-OHP levels fell progressively during the early afternoon and evening. Specimens obtained at 15-minute intervals during the last 45 to 60-minute period of sampling gave fairly consistent results in each patient.

Considerable variation was also found in the plasma 17-OHP concentration in the patient who was receiving 25 mg cortisone acetate at 6-hourly intervals. This patient showed relatively low levels between 1.00 a.m. and 3.00 a.m., with an abrupt rise during the next 2 hours. The plasma 17-OHP then remained between 8 µg/100 ml and 10 µg/100 ml until 3.00 p.m. A further value in this range was obtained at 9.00 p.m., but the remaining results appeared to show a progressive fall in the later afternoon and evening.

Discussion

Strott et al. (1969) postulated that persistently low plasma levels of cortisol in patients with CAH would lead to uninhibited ACTH secretion and that, as a result, plasma 17-OHP levels would be maintained at fairly constant high levels. The above results indicate that this is not the case and there appears to be marked circadian variation in the plasma 17-OHP level in CAH due to 21-hydroxylase deficiency. This finding is in keeping with the observations that circadian changes in plasma ACTH occur in Addison’s disease, despite persistently low plasma cortisol levels (Graber et al., 1965; Besser et al., 1971), and in normal subjects after blockade of cortisol biosynthesis by the 11-hydroxylase inhibitor, metyrapone (Jubiz et al., 1970).

We have recently tried to determine whether estimation of plasma 17-OHP can be of value in assessing treatment in patients with CAH (Barnes and Atherden, 1972). The present results indicate that the timing of such tests must be carefully standardized as estimates obtained during the late afternoon or evening may give misleadingly low values. Estimation of plasma 17-OHP may also be of some diagnostic value in patients with CAH. Our results suggest that the timing of such diagnostic tests may be less important as the plasma 17-OHP levels in all our patients remained abnormally raised during the late evening. The finding of a variation fourteenfold to eighteenfold in plasma 17-OHP indicates that the use of a single morning plasma level to calculate 17-OHP production rate (Strott et al., 1969) is likely to lead to considerable overestimation of the production rate in patients with CAH.

In patients with CAH, one of the main objectives of treatment with steroids is to suppress ACTH secretion, thereby reducing adrenal androgen secretion and preventing or reversing virilization.
The above results indicate that relatively little treatment may be needed during the evening and early hours of sleep, and it appears that the main suppressive dose of steroid is required to cover the period between 3:00 a.m. and 3:00 p.m. Our findings support the suggestion of Hamilton and Moodie (1970) that patients with CAH should be given steroids during the late evening to suppress early morning ACTH secretion. Late evening treatment may have a further advantage. It has been shown that a single dose of dexamethasone given at midnight can suppress ACTH secretion for up to 24 hours (Nichols, Nugent, and Tyler, 1965), and late night treatment of patients with CAH may permit the use of smaller doses of steroid (Hayek, Crawford, and Bode, 1971). Whether such timing of treatment can reduce the risks of steroid-induced growth retardation is still unknown. The results obtained with alternate-day steroid therapy in children with the nephrotic syndrome (Soyka, 1967) indicate that growth suppression is not solely dependent on steroid dose but may also be related to the daily timing of treatment.

We wish to thank Drs. Betty Priestley and G. H. Newns for referring their patients for investigation, and Drs. Geraldine Hill and R. Evans for help in obtaining blood samples.

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Arch Dis Child 1972 47: 602-604
doi: 10.1136/adc.47.254.602

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